Supporting information.

Rapidly Transducing and Spatially Localized Magnetofection Using Peptide-Mediated Non-Viral Gene Delivery Based on Iron Oxide Nanoparticles

Lia A. Blokpoel Ferreras[1], Sze Yan Chan[1], Saul Vazquez Reina[2] & James E. Dixon[1*]

[1]Regenerative Medicine & Cellular Therapies Division, The University of Nottingham Biodiscovery Institute (BDI), School of Pharmacy; [2]School of Veterinary Sciences; University of Nottingham, Nottingham, NG7 2RD, UK.

*Correspondence should be addressed to James E. Dixon:

james.dixon@nottingham.ac.uk

Regenerative Medicine & Cellular Therapies,
The University of Nottingham Biodiscovery Institute (BDI),
School of Pharmacy
University Park
University of Nottingham
Nottingham NG7 2RD, UK
Tel: +44 (0) 115 7486313

Figure S1.

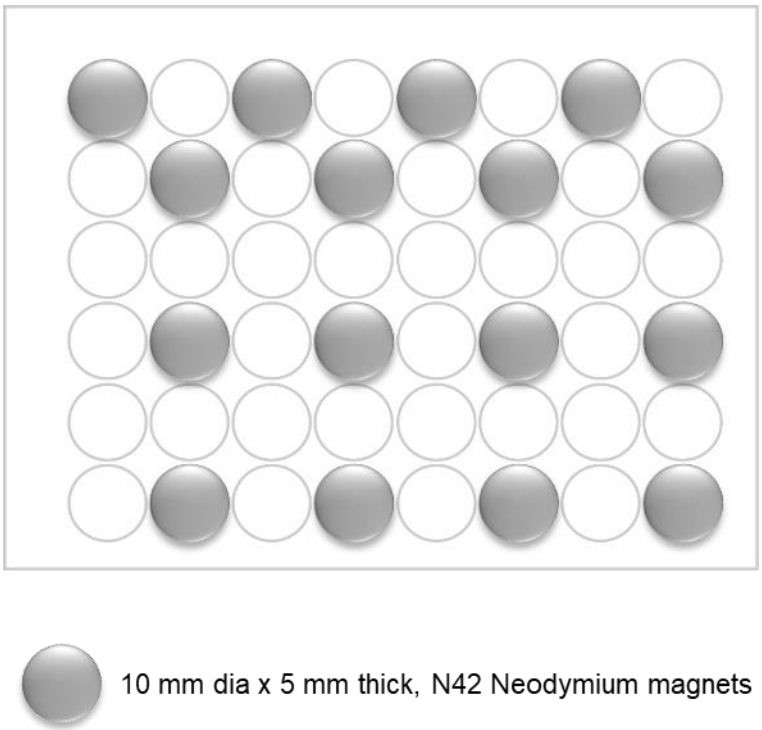

Figure S1. Magnet array configuration for magnetofection. Individual magnets were arranged and fixed in a way such that they would fit wells on a 48 well-plate format. Individual magnets were N52 Neodymium, 10 mm diameter and 5 mm thick (First for Magnets, UK catalog number F645-N52-10).

Figure S2.

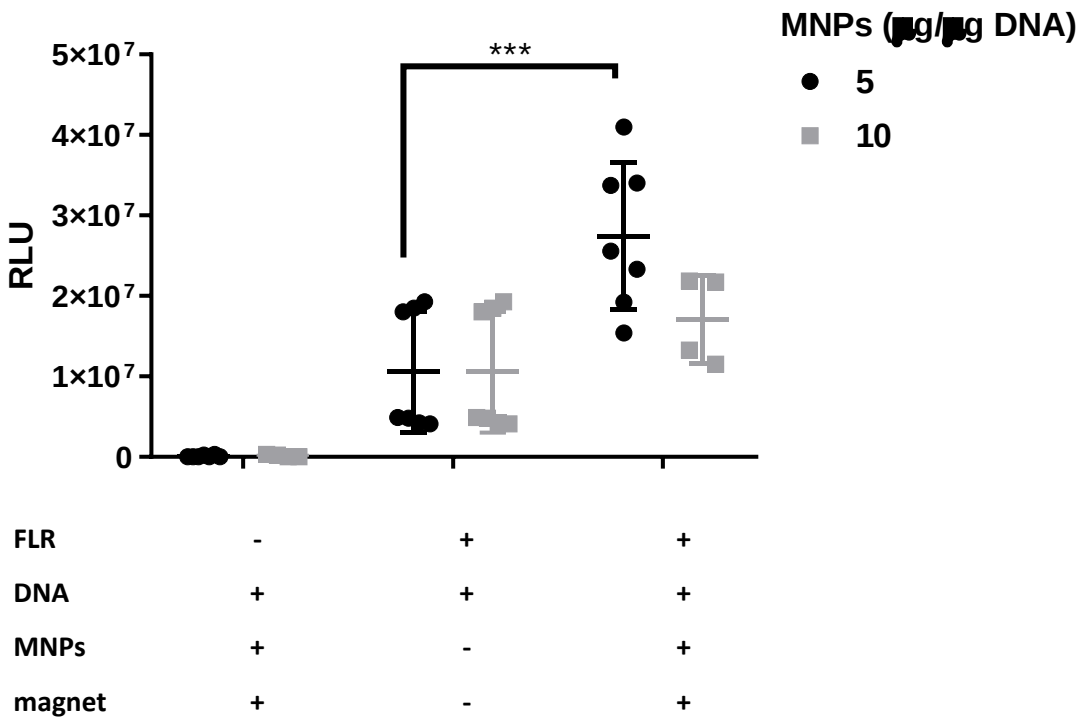

Figure S2. GET Magnetofection enhances transfection levels in NIH3t3s. Gaussia luciferase expression on NIH3t3s after magnetofection for 1 hour. DNA was delivered with MNPs (MNPs-DNA), FLR (FLR-DNA) and FLR and MNPs (FLR-MNPs-DNA). MNPs complexes were formulated at 5 and 10 µg MNPs/µg of pDNA. FLR-DNA ratio was constant at N/P 6. Graph represent mean RLU ± s.d. (n= 2-3 biological replicate, *** p>0.001, Tukey's multiple comparisons test).

Figure S3.

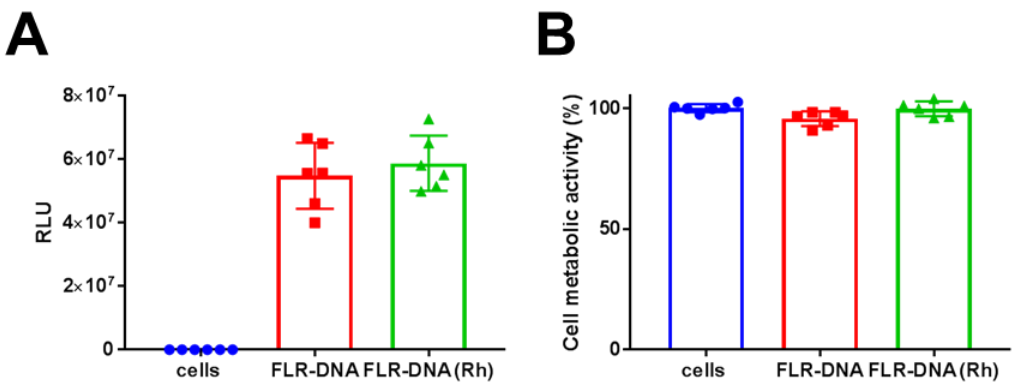

Figure S3. Rhodamine labelling of pCMV-GLuc2 pDNA does not affect its ability for protein expression or cell viability in NIH3t3 cells. A. Gaussia luciferase expression on NIH3t3 cells after 24 hours transfection with FLR and unlabelled pDNA (FLR-DNA) or FLR and Rhodamine-labelled DNA (FLR-DNA(Rh)). B. Relative cell metabolic activity of NIH3t3 cells after 24 transfection with FLR and unlabelled DNA (FLR-DNA) vs FLR and Rh-DNA (FLR-Rh-DNA) measured by Alamar Blue. Untreated cells were taken as a control for 100% cell metabolic activity. n=3, biological replicates,

Figure S4.

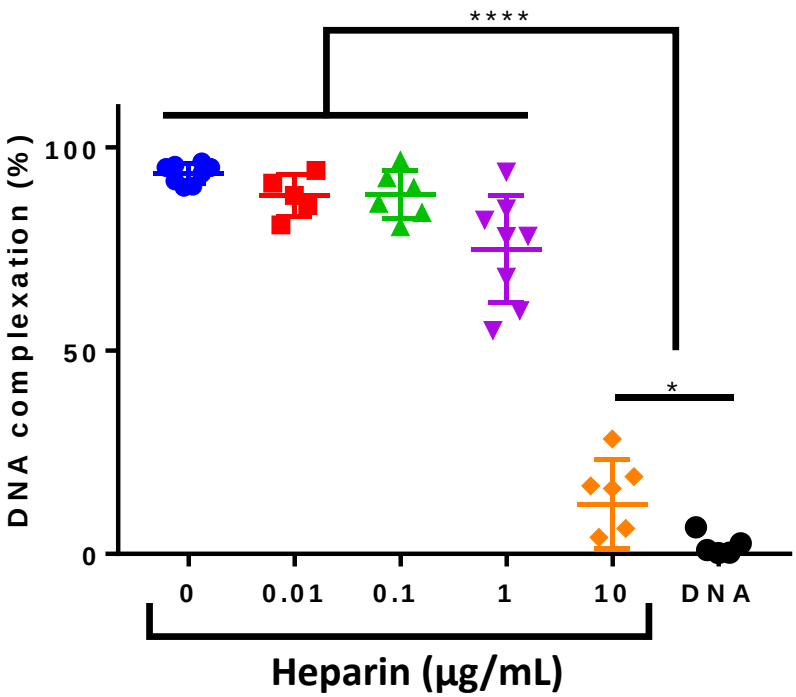

Figure S4. High concentrations of heparin destabilize the FLR-DNA complexes. Percentage of pDNA complexation with FLR at increasing concentrations of heparin: 0, 0.01, 0.1, 1 and 10 µg/mL in water. pDNA alone was used to calculate 100 % of free DNA (0% complexed pDNA). (n=3, biological replicates. *p<0.05, **** p<0.0001, Tukey's multiple comparisons test).

Figure S5.

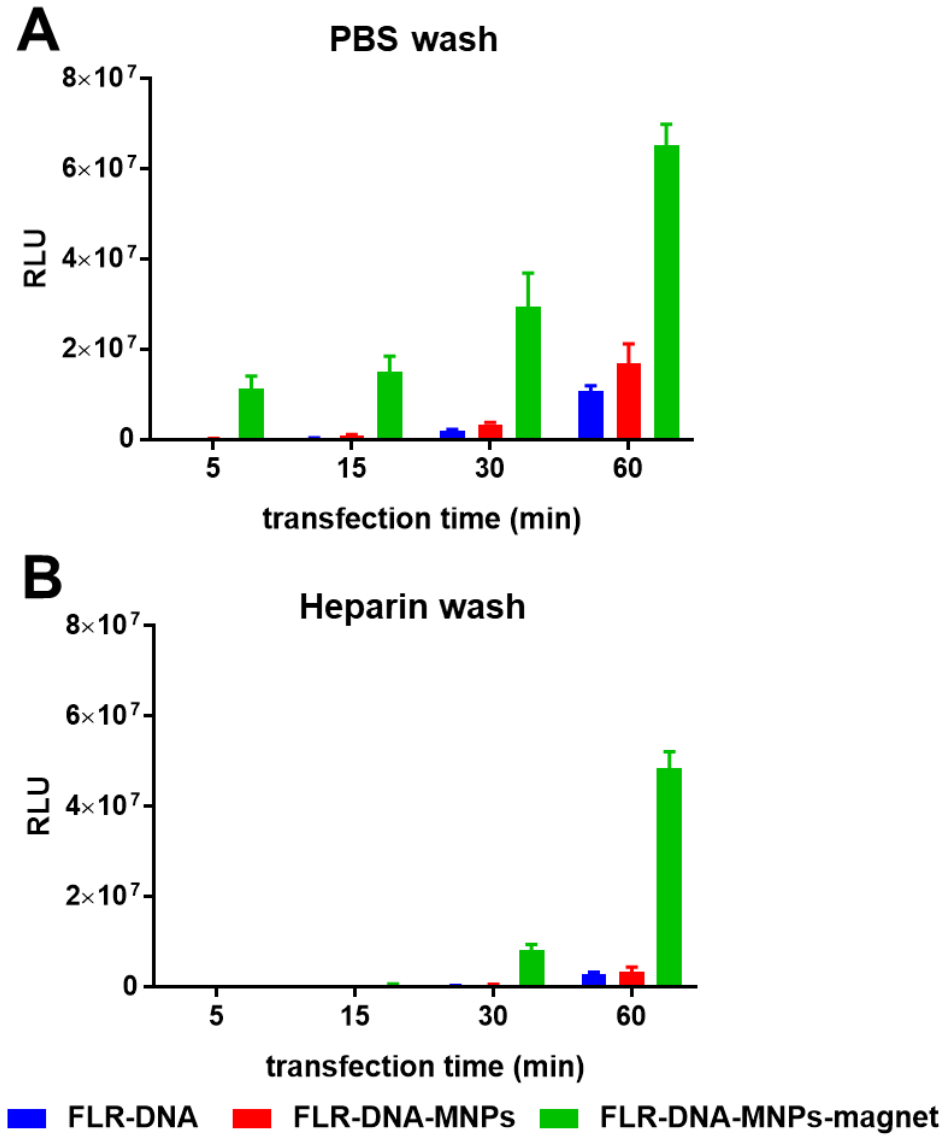

Figure S5. Efficient and fast (less than 5 min) pDNA delivery using GET magnetofection and a static magnet. Gaussia luciferase expression after 5, 15, 30 and 60 min transfection/magnetofection. After transfection cells were washed with: (A) PBS to remove any unbound DNA complex or (B) heparin to disrupt any DNA-vector complex that hasn't been uptaken. 0.5 µg of pDNA were delivered with FLR (FLR-DNA) and FLR and MNPs, FLR-DNA-MNPs with/without the application of a magnetic field. For all formulations MNPs complexes were formulated at 5 µg MNPs/µg of pDNA. FLR-DNA ratio was constant at N/P 6. n=3 biological repeats.

Figure S6.

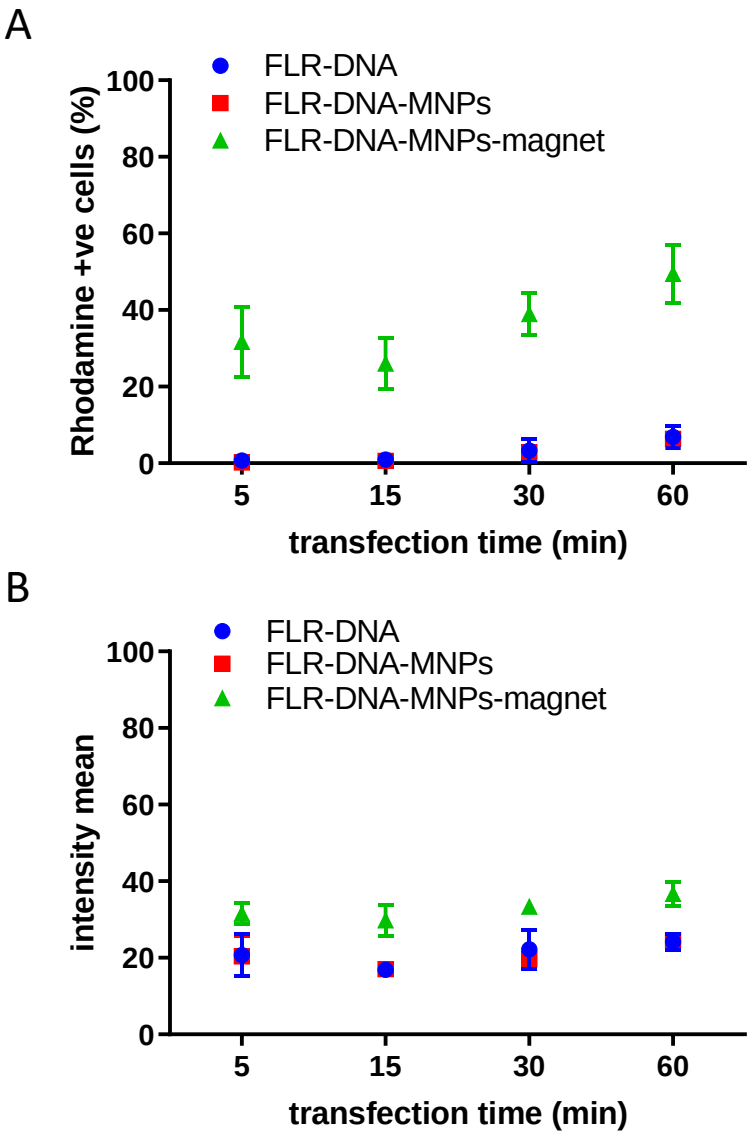

Figure S6. Efficient and fast (less than 5 min) pDNA cell membrane association using GET magnetofection and a static magnet. A. Percentage and B. Mean intensity of rhodamine (Rh) positive NIH3t3 cells after Rh-pDNA after transfection/magnetofection for 5, 15, 30 and 60 min. Rh-pDNA was delivered with FLR (FLR-DNA) and FLR and MNPs, FLR-DNA-MNPs with/without the application of a magnetic field. MNPs complexes were formulated at 5 µg MNPs/ 1 µg of pDNA. FLR-DNA N/P 6. After incubation cells were washed with heparin to remove any particles that hadn't been internalized. Values represent mean ± s.d. n=3 biological replicates.

Figure S7.

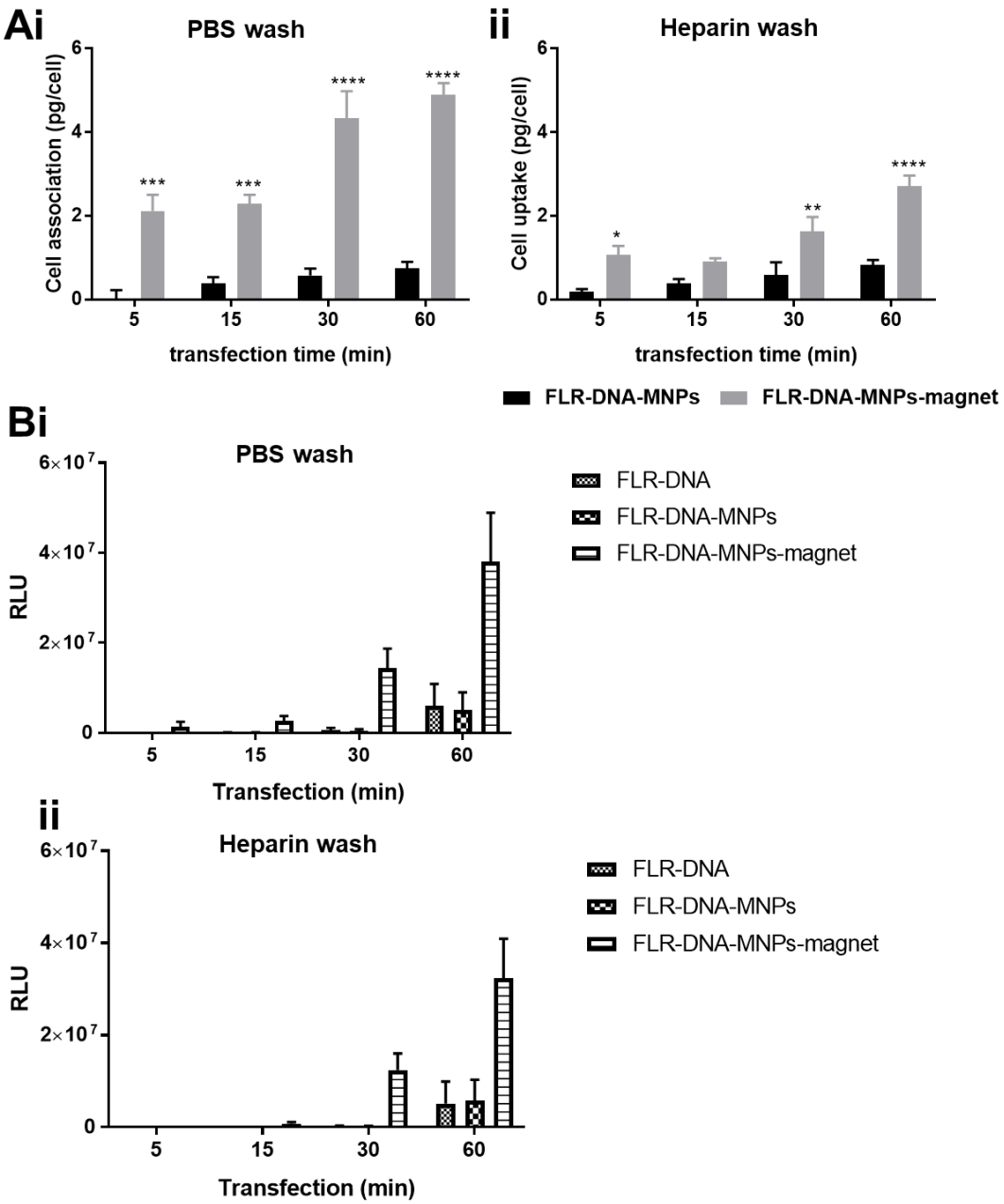

Figure S7. Rapid cell uptake within 30mins with GET magnetofection. A. Iron cell association (i) and iron cell uptake (ii) in NIH3t3s after after 5, 15, 30 and 60 min transfection/magnetofection. After transfection cells were washed with: (i) PBS to remove any unbound DNA complex or (ii) heparin to disrupt any DNA-vector complex that hasn't been uptaken. 0.5 µg of DNA were delivered with FLR (FLR-DNA) and FLR and MNPs, FLR-DNA-MNP complexes with/without the application of a magnetic field. For all formulations MNPs complexes were formulated at 5 µg MNPs/µg of DNA. FLR-DNA ratio was constant at N/P 6. n=3 biological replicates, *p<0.05, ** p<0.01, **** p<0.0001, Sidak's multiple comparisons test. B. Gaussia luciferase expression after 5, 15, 30 and 60 min transfection/magnetofection. After transfection cells were washed with: (i) PBS to remove any unbound DNA complex or (ii) heparin to disrupt any DNA-vector complex that hasn't been uptaken. 0.5 µg of DNA were delivered with FLR (FLR-DNA) and FLR and MNPs, FLR-DNA-MNPs with/without the application of a magnetic field. For all formulations MNPs complexes were formulated at 5 µg MNPs/µg of DNA. FLR-DNA ratio was constant at N/P 6. Bars represent mean values ± s.d. n=3 biological replicates.

Figure S8.

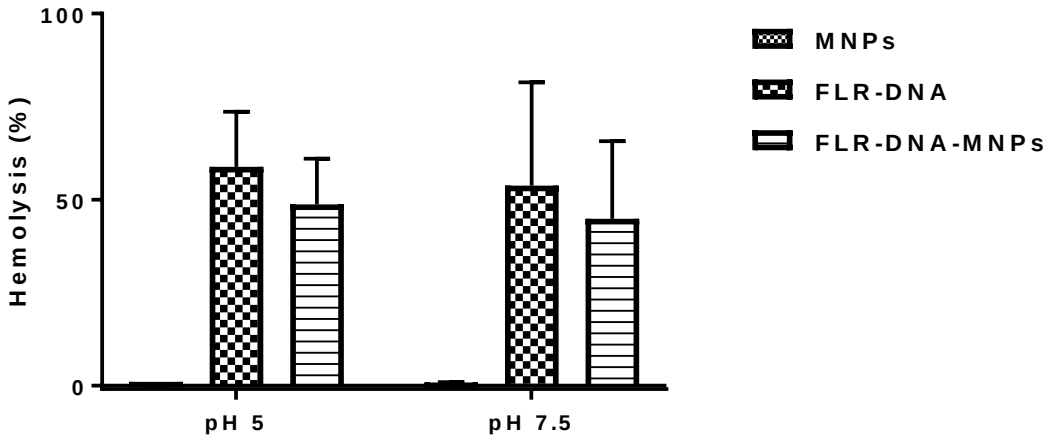

Figure S8. Membrane rupturing activity of GET Magnetofection is mediated by FLR and independent of pH. Freshly isolated human erythrocytes were exposed to 0.5 µM of FLR. For FLR-DNA, N/P ratio 6 between peptide and DNA. 5 µg of MNPs/µg of DNA were added to form the FLR-DNA-MNPs. Haemolysis experiments were performed in PBS for 30 min at physiological pH (pH 7.5) and late endosome pH (pH 5). Haemolysis was quantified by spectrophotometric assay. Results are expressed as percentage lysis taking Triton-X 100 as complete lysis (100%). Bars represent mean ± s.d. from 7 independent repeats from the same donor.

Figure S9.

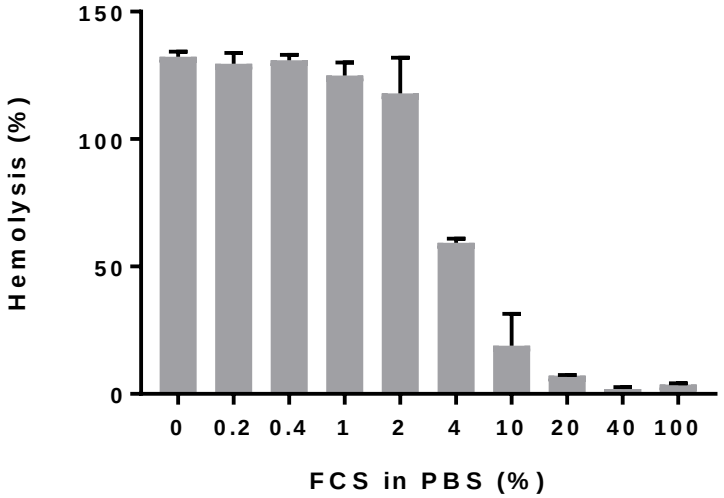

Figure S9. FLR minimally affects membrane integrity in cell culture conditions. Freshly isolated human erythrocytes were exposed to 1 µM of FLR at increasing concentrations of FCS (0-100% v/v in PBS). Hemolysis experiments were performed for 30 min at physiological pH (7.5). Hemolysis was quantified by spectrophotometric assay. Results are expressed as percentage lysis taking Triton-X 100 as complete lysis (100%). Bars represent mean ± s.d. from 2 independent repeats from the same donor.

Figure S10.

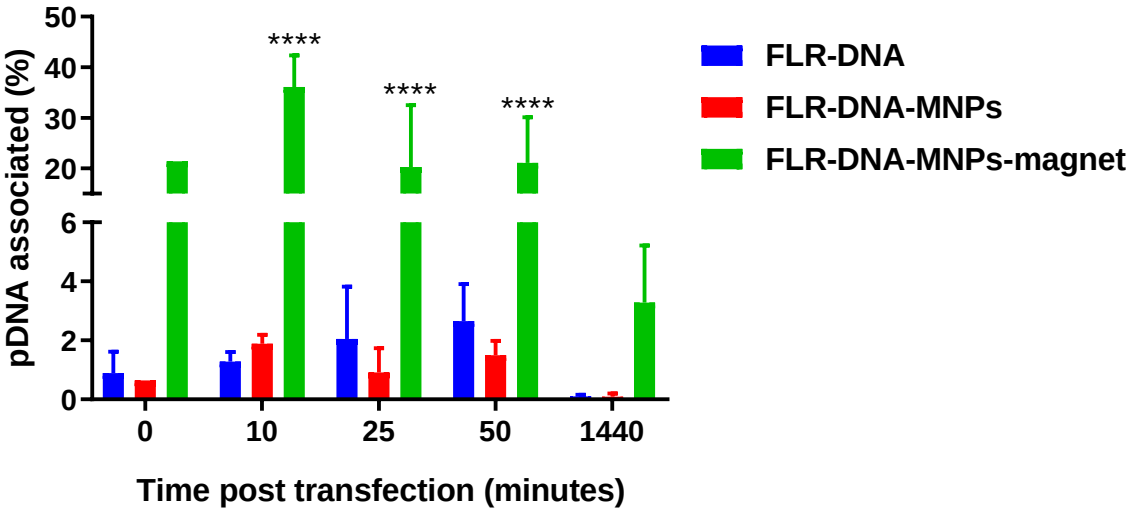

Figure S10. Stability of GET Magnetofected pDNA. Percentage of intact plasmid DNA associated with NIH3t3 cells after 5 minutes transfection/magnetofection. Unbound pDNA was washed with PBS. Collection points were: immediately after (0 min), 10, 25, 50 and 1440 min. 0.5 µg of pDNA were delivered with FLR (FLR-DNA) and FLR and MNPs, FLR-DNA-MNPs with/without the application of a magnetic field. MNPs complexes were formulated at 5 µg MNPs/ 1 µg of pDNA. FLR-DNA ratio was constant at N/P 6. Extrachromosomal pDNA was extracted, purified and quantified by bacterial transformation. Percentage of pDNA associated was calculated from the total amount of DNA delivered. Bars represent mean percentage of cell associated DNA ± s.d. (****p<0.0001 compared to FLR-DNA-MNPs, Tukey's multiple comparisons test, n= 3 technical repeats).